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Research Article

RESPONSE OF PIGEONPEA (*Cajanus cajan* L. Millsp.) GENOTYPES UNDER WATER LOGGING STRESS

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Abstract- Water logging stress was imposed on two pigeonpea genotypes *viz.*, ICPL-84023 and DA-11. Thirty days old pot grown plants were subjected to water logging continuously for 4 and 6 days in water filled containers and water was maintained 4-5 cm above the surface of soil. Significant genotypic differences were observed with respect to biochemical changes in leaves and roots. Chlorophyll content was found more in ICPL-84023 genotype as compared to DA-11 in control condition. After imposition of water logging stress ICPL-184023 genotype performed better as compared to DA-11 genotype. ICPL-84023 genotype showed less chlorophyll reduction, increased activity of antioxidant enzymes peroxidase, catalase, superoxide dismutase and ascorbate peroxidase, and less H_2O_2, MDA content as well as less cell membrane leakage. In this study DA-11 was observed as susceptible and ICPL-184023 as tolerant genotype under water logging stress.

Keywords- Pigeonpea, Water logging and Anti-oxidative Mechanism.

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Introduction

Waterlogging causes severe loss in crop production and productivity in India. Out of the total (3.9 million hectares) area under pigeon pea, about 1.1 million hectares is affected by excess soil moisture and it causes an annual loss of 25-30% in crop production [1]. In India, pigeonpea is mainly grown in mean annual rainfall between 600 and 1500 mm. Water logging condition has emerged as one of the major production constraint of Pigeonpea cultivation in pigeonpea growing states of India *viz.* Uttar Pradesh, Madhya Pradesh, Gujrat, Andhra Pradesh, Maharashtra, Karnataka and Bihar in recent years (Proceedings of the NFSM-funded project, June 2011). Areas which are dependent on monsoon are more prone to water logging. Water logging occurs when rainfall or irrigation water is collected on the soil surface for prolonged periods without infiltrating into the soil. Water logging is defined as prolonged soil saturation with at least 20% higher than the field capacity [2].

Water logging condition leads to reduce gas exchange between plant tissue and atmosphere [3]. It causes oxygen deprivation in soil and reduces availability of oxygen to roots [4]. Nutrient deficiency is the major cause of poor plant growth in waterlogged soil [5]. Water logging condition induces hypoxia (Low oxygen) and later creates anoxic (Complete absence of oxygen) condition. Prolonged water logging condition causes inhibition of root respiration and reduction in energy production. To cope energy requirement, plant shifts to fermentation mechanism [6]. Water logging generates oxidative stress and promotes production of reactive oxygen species (ROS) which cause detrimental effects on plants [7]. One of the metabolic activity affected by water logging is antioxidant system in plant. Plants have developed the defense system to mitigate the oxidative damage by increasing activity of antioxidant enzymes such as superoxide dismutase, catalase, various peroxidases, glutathione reductase which effectively scavenge the ROS and limits the ROS production [8]. The present study was done to investigate the response of Pigeonpea genotypes under short term water logging condition.

Material and Methods

Plant material and Treatment-

Disease free and healthy seeds of Pigeonpea (*Cajanus cajan L.*) genotype DA-11 and ICPL-184023 was procured from the Department of Genetics & Plant Breeding, Institute of Agriculture Sciences; B.H.U., Varanasi. Soil was collected from Experimental Farm, Institute of Agricultural Sciences, B.H.U. It was cleaned by removing the stones; weeds etc. and the soil to be used in the pots were dried, powdered and mixed thoroughly. Soil, sand and FYM were mixed in the ratio of 2:1:1. Plastic pots of diameter 9.5 cm were taken and filled with 750 g mixed soil mixture. Fertilizer was applied at the ratio of 25:50:0 ppm N: P: K pot^{-1} respectively to the pots two days before sowing. Soil was irrigated with tap water.

Water logging Treatment-

After one week of sowing, three healthy and uniform seedlings were maintained in each pot. Water logging stress was imposed 30 days after sowing. Plants were kept in this condition for four and six days. For creating waterlogged condition, plastic pots were placed in water filled bigger plastic containers, in such a way that the pots were completely submerged and water level in the container was maintained 4-5 cm above the soil surface in the pots. This water level was maintained daily. For comparison other set of pots were maintained at optimal supply of soil moisture, and these were termed as 'control'.

Sampling and observation-

Observations pertaining to biochemical parameters were recorded on control and waterlogged plants at 4 and 6 days after imposing water logging stress. Samples were collected between 9.00 to 10.00 AM. Upper most fully expanded leaf was brought to the laboratory in ice buckets. For collecting root samples, plants were uprooted carefully and soil was washed out. Sample of leaves and roots were taken and folded in aluminum foil and dipped in liquid N_2. After 24 hours, the

samples were withdrawn from liquid N_2 and stored in deep freezer (−60°C) for further analysis. Biochemical parameters were analyzed as soon as possible after storage.

Physiological assays-

Among biochemical parameters chlorophyll content, malondialdehyde (MDA) content, hydrogen peroxide (H_2O_2), cell membrane injury and enzymatic activities of catalase (CAT), peroxidase (POD), ascorbate peroxidase (APX) and superoxide dismutase (SOD) were recorded at four and six days after water logging. Chlorophyll content in the leaf samples was determined by the method as prescribed by [9]. Malondialdehyde (MDA) content was determined in root and leaf samples in normal and waterlogged plants. The level of lipid peroxidation was estimated as the MDA content and determined according to the method of [10]. Method is described in detail by[11]. The rate of H_2O_2 production was estimated spectrophotometrically in leaf and root sample of Pigeon pea using Titanium Sulphate by following method of Mukherjee and Choudhari [12]. Activities of antioxidant enzymes SOD (1.15.1.1), POD (1.11.1.7) and APX (1.11.1.11) were estimated by the method described by [13]. Activity of CAT (1.11.1.6) was estimated by the method of Beers and Seizer [14]. Cell membrane injury was calculated in root and leaf samples after 4 and 6 days of imposing water logging stress in normal and waterlogged plants [15]. Per cent membrane injury was calculated as: Per cent membrane injury = 100 - [1 - (C1/C2) × 100].

Statistical Analyses-

Completely Randomized Design was followed and analysis of variance was performed on the data as described by Panse and Sukhatme [16]. Critical difference values were calculated at 1 percent level of significance in order to compare treatment means.

Results

Visual symptoms of water logging stress were observed as yellowing of leaves. Chlorophyll content of both genotypes were decreased significantly during water logging stress. Decrease of 13.8 and 18.9 percent of total chlorophyll content was observed in 4 days and 6 days water logging stressed ICPL-84023 plants respectively as compared to control. In genotype DA-11, 20 and 25.5 percent decrease was observed in 4 days and 6 days water logging treatment respectively. Decrease in chlorophyll content is less in genotype ICPL-84023 as compared to genotype DA-11 [Table-1]. Water logging stress treatment increased the activity of SOD, CAT, APX, and POX in leaf [Table-1] and root [Table-2] samples of both genotypes. Percentage increase in activity of SOD and POX was observed more in root tissue as compared to leaf. Increase of 29.1 and 50.4 percent of SOD activity was observed four days and six days after Water logging in root tissue of genotype ICPL-84023 while 6.7 and 17.3 percent increase was observed in root tissue of genotype DA-11 as compared to their respective control. POX activity was increased 205 (2 fold) and 266 percent (2.5 fold) in root tissues of 4 and 6 days water logging stressed plants of ICPL-184023 genotype. Activity of CAT and APX was observed more in leaf tissues after water logging treatment in ICPL-184023 genotype. DA-11 genotype showed less increase in activity of antioxidant enzymes and increased membrane damage was observed by MDA content, H_2O_2 content and cell Membrane injury [Table-1] and [Table-2]. Three fold and 5 fold increase of cell membrane injury in percentage was observed in DA-11 leaf tissues after 4 and 6 days stressed plants [Table-1]. MDA content was increased of around 196.8 and 125 percent in root tissue of 4 and 6 days stress plants respectively [Table-2].

Table-1 *Effect of short term water logging in leaves of two genotypes of Pigeonpea*

Genotype	Treatment	Chl (mg g^{-1} Fwt)	SOD (EUg^{-1} Fwt m^{-1})	CAT (EUg^{-1} Fwt m^{-1})	POX (EUg^{-1} Fwt m^{-1})	APX (EUg^{-1} Fwt m^{-1})	H_2O_2 (µM g^{-1}Fwt)	MI (%)	MDA content (nmol g^{-1}Fwt)
ICPL-84023	Control 4	2.59	30.54	21.33	2.37	21.55	3.92	4.06	1.42
	WL 4	2.23 (-13.8)	38.55 (+26.2)	35.51 (+66.5)	5.52 (+132.9)	25.26 (+17.2)	5.55 (+41.5)	5.56 (+36.9)	2.98 (+37.8)
	Control 6	2.69	31.84	19.85	2.58	20.43	4.06	4.26	1.57
	WL 6	2.18 (-18.9)	39.09 (+22.7)	39.60 (+99.5)	5.59 (+116.7)	28.72 (+40.5)	6.27 (+54.4)	7.31 (+71.6)	2.74 (+74.5)
DA-11	Control 4	2.10	29.77	21.11	2.56	20.89	3.87	4.48	1.29
	WL 4	1.68 (-20)	32.55 (+9.3)	25.83 (+22.4)	4.66 (+82.0)	22.52 (+7.8)	6.33 (+63.5)	18.44 (+311.6)	3.60 (+179.1)
	Control 6	1.92	28.16	19.98	1.88	21.21	3.81	4.52	1.90
	WL6	1.43 (-25.5)	33.96 (+20.5)	24.86 (+24.4)	4.90 (+160.6)	22.02 (+3.8)	7.44 (+95.3)	28.18 (+523.4)	3.54 (+86.3)
	SEm±	0.06	0.26	0.28	0.12	0.36	0.09	0.08	0.06
	CD@1%	0.23	1.06	1.14	0.44	1.67	0.37	0.32	0.24

Figures in parentheses indicate percentage increase and decrease under water logging condition over their respective control.
Control 4: Control (optimum moisture condition/No water logging), for comparison with 4 days water logging stressed plants.
Control 6: Control (optimum moisture condition/No water logging), for comparison with 6 days water logging stressed plants.

Table-2 *Effect of short term water logging in roots of two genotypes of Pigeonpea*

Genotype	Treatment	SOD (EUg^{-1} Fwtm^{-1})	CAT (EUg^{-1} Fwtm^{-1})	POX (EUg^{-1} Fwtm^{-1})	APX (EUg^{-1} Fwt m^{-1})	H_2O_2 (µMg^{-1} Fwt)	MI (%)	MDA content (nmol g^{-1}Fwt)
ICPL-84023	Control 4	25.03	17.62	1.53	10.53	1.13	4.36	0.95
	WL 4	32.35 (+29.2)	25.12 (+42.6)	4.68 (+205.9)	11.44 (+8.6)	1.69 (+49.5)	7.76 (+77.9)	1.68 (+76.8)
	Control 6	24.26	17.07	1.48	10.71	1.50	4.44	1.26
	WL 6	36.51 (+50.4)	29.20 (+71.1)	5.42 (+266.2)	13.97 (+30.4)	2.30 (+53.3)	8.30 (+86.9)	1.58 (+25.3)
DA-11	Control 4	25.23	17.69	1.48	10.74	1.54	4.89	0.95
	WL 4	26.94 (+6.7)	18.48 (+4.5)	2.52 (+70.3)	11.42 (+6.3)	3.71 (+140.9)	19.96 (+308.1)	2.82 (+196.8)
	Control 6	24.46	21.56	1.43	10.83	1.51	4.76	1.32
	WL6	28.71 (+17.3)	25.67 (+19.1)	2.79 (+95.1)	11.31 (+4.4)	4.60 (+23.9)	22.65 (+375.8)	2.98 (+125.7)
	SEm±	0.17	0.15	0.09	0.12	0.10	0.11	0.02
	CD@1%	0.69	0.62	0.35	0.44	0.42	0.43	0.09

Figures in parentheses indicate percentage increase and decrease under water logging condition over their respective control.
Control 4: Control (optimum moisture condition/No water logging), for comparison with 4 days water logging stressed plants.
Control 6: Control (optimum moisture condition/No water logging), for comparison with 6 days water logging stressed plants.

Discussion

Reduction in total chlorophyll content in leaf tissues has been observed under waterlogged condition in pigeonpea genotypes [17], barley[18], mungbean [19], soybean [20] field bean [21] and tomato [22]. The reason of loss of chlorophyll content is reduction in water and nutrient uptake under water logging condition. Nitrogen and Magnesium elements are components of chlorophyll structure and reduction in uptake of these elements reduce the chlorophyll content in leaves [23, 24]

It is well understood that under abiotic stresses, such as waterlogging, there is increase in the levels of reactive oxygen species (ROS).Resistant plant genotypes have efficient defense mechanism, involving enzymes, *viz.,* peroxidase (POX), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione reductase (GR) and non-enzymatic constituents, *viz.,* ascorbate, glutathione etc. to detoxify ROS[25]. Superoxide dismutase (SOD) catalyzes the dismutation of superoxide radical, which is highly toxic to plants. During water logging, increased activity of SOD was reported in barley [26] citrus [27] and pigeonpea [28, 29]

High activity of SOD increases more hydrogen peroxide concentration but this is harmful for cell. There are several enzymes in plant system which converts this hydrogen peroxide to water. Catalase is the important enzyme which catabolizes the hydrogen peroxide to water in very less time. Other enzymes which detoxify the peroxide are POD and APX etc. It was reported that during post anthesis water logging, there was increase in POX activity in water logging resistant wheat genotype [30]. In barley, increased POD activity under flooding condition was reported; wherein, it was suggested to play important role in ROS scavenging [31, 32]. Thus increase activity of SOD, POX, CAT and APX reduces the ROS and alleviates the oxidative stress caused by water logging condition.

Conclusion

Water logging causes oxidative damage by increasing production of ROS. Antioxidant system is developed as defense mechanism in plant against oxidative stress. Increased activity of SOD, CAT, POX and APX was observed in plants under stress. ICPL-184023 showed higher increase in antioxidant enzyme activity in both leaves and roots as compared to DA-11 genotype. Less membrane damage observed where antioxidant system was more pronounced. Direct relationship was observed between malondialdehyde (MDA) contents and cell membrane injury. The MDA content as well as cell membrane injury was the maximum at 6th day after imposing stress in both the genotypes and its magnitude was very high in genotype DA-11. It was also concluded that either root MDA content or cell membrane injury may be taken as parameter to screen out water logging resistant pigeonpea genotypes. Cell membrane injury, hydrogen peroxide and MDA content was observed lower in genotype ICPL-84023 as compared to DA-11 and hence ICPL-84023 genotype was observed tolerant genotype and DA-11 as susceptible genotype.

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